THE MEIOTIC CHROMOSOMES OF THE MALE LLAVEIELLA TAENECHINA MORRISON (COCCIDAE) AND THE QUESTION OF THE TERTIARY SPLIT

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The meiotic chromosomes of the male *Llaveiella taenechina* present a new type of observational evidence on the old question of the time of chromosome division. In spite of the overwhelming evidence now accumulated in favor of a multiple strand structure at all stages of both somatic and meiotic chromosomes, there is still disagreement as to the exact number present at any point in the chromosome cycle. Recent reviews of the extensive literature on this question are available (Lorbeer, 1934; Kaufmann, 1936; Geitler, 1938). Suffice it here to say that the vast majority of evidence and of observers supports the idea of a somatic anaphase chromosome at least double in structure which reduplicates in pro- or early metaphase to form a four-parted metaphase chromosome. The last spermatogonal division differs in no way from the preceding one and the meiotic chromosomes are thus theoretically, even if not always visibly, double at leptotene, four-parted at metaphase, and form eight-parted tetrads. The older idea of univalent leptotene chromosomes reduplicating at pachytene to give four-parted tetrads is maintained by few among recent writers, among them Geitler (1938) and Darlington (1937). The persistence of this concept in the face of overwhelming evidence to the contrary stems partially from the reluctance of geneticists to abandon Darlington's precocity theory of meiosis. This hypothesis, as Huskins (1937) has pointed out, has proved stimulating and valuable but its basic assumptions must now be admitted to be untenable. A conservative point of view toward the evidence for multiple strand structure stems also from the technical difficulties in the way of its demonstration; admittedly it cannot be demonstrated for all stages of the cycle nor in all organisms.

In the meiosis of the male *Llaveiella taenechina* the multiple structure of the chromosome is rendered dramatically visible by a unique autonomy of the individual chromatids in their metaphase behavior and their ana-

phasic movements. Not only is the tertiary split [1] present, but its reality is demonstrated beyond possibility of cytological misinterpretation by the fact that the pairs of half chromatids separated by it proceed in their poleward migration at individually different rates. Two other phenomena of the *Llaveiella* meiosis contribute to the demonstration of the tertiary split—first, the high incidence of asynapsis among the chromosomes, and second, the tendency for the equational halves of the sex chromosome to separate and to divide independently of each other. The possibility of resolving a chromosome optically into its constituent units is obviously increased by subdividing the mass—as in the cases of the asynaptic chromosomes and the separate X chromatids—and by the dissociation of the larger unit by the differential rates of movement among its components.

Material and Methods

Llaveiella taenechina belongs to the most primitive tribe (Llaveiini), and sub-family (Monophlebinae) of the coccid family Margarodidae. The insects were collected in the vicinity of Ixtepec, Oaxaca, Mexico, from their favorite host plant the cascalote, *Caesalpinia coriaria.* Fertilized eggs were transported to New York and the young were raised to maturity in the Columbia University greenhouse. Over 150 males of all ages were available for the present study. Considerable data on the biology of this little-known species have been accumulated and will be published elsewhere. For the purposes of this report I need only say that meiosis in the male occurs during the third and fourth nymphal instars. The testes were dissected out in Allen's modification of Bouin's fixative, sectioned at 4, 5, 6 and 7 micra, and stained with Heidenhain's haematoxylin. Other methods (Benda, Flemming fixations, Feulgen, etc.) were used to check results but the present report is based on the Bouin-haematoxylin preparations.

Acknowledgments

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[1] The term "tertiary split" refers to the division of the meiotic chromosomes preparatory to the first post-meiotic mitosis—the unused cleavage of the meiotic mitoses. The name "tertiary" derives from the old identification of the plane of contact between paired homologous chromosomes as the primary split (reductional plane of the tetrad in the old terminology), while the split effective in the division of the chromosomes at meiosis (the equational plane of the tetrad) was known as the secondary. The next division plane was accordingly termed the tertiary split.

of the New York office for their continued coöperation. I am also deeply indebted to Drs. J. M. and A. C. Baker of Mexico City for continued assistance in the collection and transport of the insects. The field work during 1938 was supported by a grant from the Carnegie Corporation to Professor Franz Schrader of Columbia University.

General Account of Meiosis

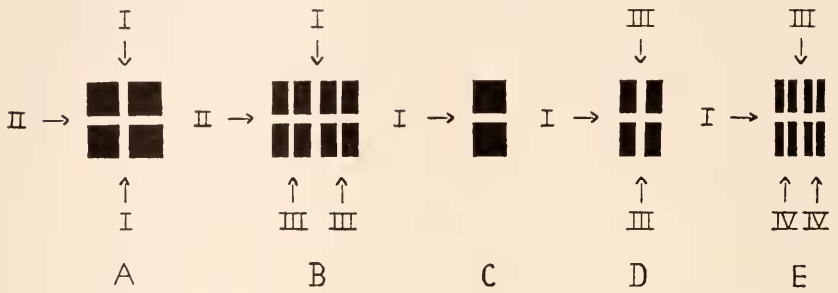
The chromosome complement of the *Llaveiella tacnechina* ♀ comprises 3 pairs of rod-shaped chromosomes differing in length—one long, one slightly shorter, and one very short pair (Fig. 1). In the the ♂ the shortest chromosome is unpaired giving a complement of 5 (Fig. 2). In its general features meiosis parallels that found in the genus *Llaveia* (Hughes-Schrader, 1931). The chromosomes are the same in number in the two genera; the autosomes, moreover, show very comparable size relations as well as some strikingly similar peculiarities of behavior. The sex chromosomes of *Llaveia*, however, constitute the largest pair,—much longer than either pair of autosomes,—while in *Llaveiella*, they are very short,—much shorter than any of the *Llaveia* set. While of course not conclusive, these relations suggest that the X chromosome of *Llaveiella* is derived from the *Llaveia* X by loss or losses—rather than through any transformation of autosome into sex chromosome. The derivative relationship of *Llaveiella* to *Llaveia* is, moreover, borne out by the detailed study of the cytology of the two forms.

Somatic and spermatogonial mitoses are orthodox both as regards chromosomes and achromatic figure. The meiotic prophase is initiated with the growth and lobulation of the nucleus into 3 separate vesicles proportional in size to the chromosome pairs. In each of the two larger vesicles one pair of the longer chromosomes (autosomes) evolves and passes through characteristic leptotene, zygotene, pachytene, and diplotene stages. In the small vesicle the sex chromosome, visibly split since an early stage, condenses into what appears in cases of maximum contraction to be a bivalent body. The vesicle walls then disappear in situ; each tetrad and dyad concomitantly gives rise to individual half spindle elements. As in *Llaveia*, these half spindles have no central bodies and their fibers diverge in an outflaring truncated cone, instead of converging to a center. At first devoid of common orientation, these individual spindles gradually orient themselves into parallel position, with the chromosomal masses in a metaphase plate arrangement. Figure 3 shows such a typical metaphase—with the chromosome elements at maximum contraction. Figure 4 gives the polar aspect of a similar plate. The two tetrads seem each four-parted—two-parted in polar view—the X

chromosome two-parted, with one element only visible from the end. The slightly pointed projections of the chromosome ends, continuous with the half-spindle substance, represent a thin flange or collar of spindle material which seems to be drawn out from the chromosome or to draw out the chromosome sheath as it forms—and do not indicate subdivision of the chromosome. This is clear from the fact that the profile outline remains the same from whatever position the chromosome and its spindle be viewed. Anaphasic movement starts with the compression and elongation of each of the chromosomal elements. An individual, tubular interchromosomal element or stembody, continuous with the chromosome sheath and the boundary wall of the half spindle, grows between the separating chromosome masses—which are progressively constricted like drops in a capillary tube as they pass to the poles (Fig. 5). An interesting variation from the *Llaveia* figure lies in the very early appearance of the cytoplasmic furrow which cuts through the individual stembodies without visibly affecting their structure or position. The sex chromosome divides equationally in this first division and both telophase nuclei in all cases of maximum contraction and synchrony of chromatid movement,—show the same chromosome group of five elements,—two chromatids from each tetrad, one from the X dyad. A short interkinesis with a variable degree of chromosome loosening follows (Fig. 6)—after which the chromosomes recondense. The homologous chromatids then re-associate; and each dyad thus formed gives rise to its own half spindle. The X, usually with some indication of doubleness, produces no half spindle and moves as a passive body to one pole. Division of the dyads proceeds as in first anaphase with the formation of individual stembodies which may later fuse into a single tube during late anaphase.

The foregoing condensed account is based on cells showing the maximum condensation of chromosomes and the maximum synchrony among their elements. It parallels *Llaveia* (*Llaveia bouvari,* Hughes-Schrader, 1931, *Llaveia oaxacaensis,* and an as yet undescribed genus of the Llaveiini, unpublished data Hughes-Schrader) ; is ancestrally rather than derivatively related to the *Protortonia* type (Schrader, 1930) ; and probably represents the basic meiotic pattern for the tribe Llaveiini. Actually, however, in *Llaveiella taenechina* this basic pattern is realized in only a small percentage of the spermatocytes. The variations far outnumber the regular cases. The variations are of four basic types—expressed to different degrees and in different combinations in different cells. As indicated above, these variant procedures involve: (1) chromatid autonomy and differential rates in chromatid separation and anaphasic movements, (2) separation and independent division of the equa-

tional halves of the sex chromosome, (3) asynapsis, and (4) the occasional further subdivision of the chromosome along the fourth or quarternary split. I would especially emphasize that all these variants lie within the normal range of meiotic procedure for this species. The evidence for this is convincing. All the variant procedures culminate in a regular reduction of the chromosomes. There is a striking absence of degenerating cells or cysts in the testes of the many males studied; all the visible evidence shows that the variant types are successful in sperm formation. Breeding data further support this conclusion. There is no loss of eggs after fertilization and the percentage of hatching and the viability throughout development is amazingly high. A description of the variant procedures follows.



Text Fig. 1. *A*. Tetrad of *Llaveia*-type with primary (I) and secondary (II) splits. *B*. Tetrad of *Llaveiella*-type, showing an additional, tertiary split (III). *C*. Second division chromosome of *Llaveia*-type, with only the primary split (I) visible. *D*. Second division chromosome of *Llaveiella*-type, with primary (I) and tertiary (III) splits. *E*. Same as *D* with addition of quarternary (IV) split, as occasionally found in *Llaveiella*.

Chromatid Autonomy in Metaphase Behavior and Division Rate

First Division

The tertiary split becomes clearly evident in the majority of first metaphase chromosomes. Only rarely do these show the compact association of two chromatids per chromosome, four per tetrad, of the tribal type (Figs. 3 and 4). Far more frequently the association of the four chromatids in the tetrad is a loose one; they slide upon one another and take up various positions in relation to each other. Thus separated the tertiary split in each becomes clearly evident and, as in Fig. 8, each tetrad is seen to be obviously eight-parted.

The identification of the planes of separation in this and the following figures will be facilitated by reference to Text Fig. 1. In *A* the compact tetrad of the *Llaveia* type is shown. The distinction between

the primary and secondary splits (I and II) is, of course, arbitrary and meaningless in cases where crossing over has combined the reductional and equational planes. The identification here used is based on cases of asynaptic chromosomes whose orientation is similar and in which the secondary, and in this case truly equational, split is the one effective in the first division. *B* shows the *Llaveiella* tetrad, subdivided along the tertiary split III—which is thus seen to separate pairs of half chromatids —each pair thereupon assuming autonomy in spindle formation.

Resuming the analysis of Fig. 8,—it is evident that the pairs of half chromatids separated by the tertiary split retain a closer association than do the pairs of whole chromatids. All four pairs of half chromatids may line up parallel to each other in a single row, as in Fig. 8 (a polar view of this arrangement is shown by one tetrad in Fig. 9), or the two quartets may lie at right angles to each other—as shown by both tetrads of Fig. 12 and by one tetrad of Fig. 11. Again the two quartets may lie side by side in two rows giving the compact plate of four elements in end view, as in both tetrads in Fig. 10 and one in Fig. 9. The half spindles reflect to some degree this subdivision and separation of the chromosome elements which seem to give rise to them, but lamination of the half-spindle substance is restricted to the chromosome end of each half spindle. At its distal end the spindle substance of the separate elements seems to fuse into a single flaring, truncated cone (see the stippled end views of cones in Fig. 10).

Even more dramatic evidence for the tertiary split is available in the anaphasic behavior of these chromosomes. This stems from the fact that anaphasic separation and poleward movement of the pairs of half chromatids may proceed at very different rates. Very frequently one pair of half chromatids within a tetrad group starts its anaphasic separation in advance of the others (Figs. 13 and 14). Two factors in chromosome movement may be differentiated here; one, apparently intrinsic to the chromosome, expresses itself in a lengthening of the chromosome elements along the longitudinal axis of the spindle; the second is the formation and growth of the interzonal connective or stembody. If, as appears probable, half-spindle wall and stembody are simply extensions of a chromosome sheath, both effects may be produced by the elongation and thinning of the one tubular sheath with a consequent compression of the chromosomal material. But since changing shape (elongation and thinning) so often precedes any formation of stembody, I am inclined to think that two factors operate here. In the tetrad of Fig. 13 one chromatid pair is dividing precociously; its elements have elongated and it has already formed an individual stembody between them, while the two laterally placed pairs of half chromatids

show some change in shape but no stembodies are as yet visible. In the foreground tetrad of Fig. 14 the two paired elements nearest the observer show equal stembody formation between their chromosome masses, while the change of shape factor is precociously expressed in the left-hand chromatid only. In this tetrad, then, we have one chromatid pair precociously elongated and with an initial stembody formation—one chromatid, in the background, which shows neither elongation nor stembody—while the other two chromatids are dividing synchronously with a common stembody and very little elongation of their chromosome elements. Even here, however, the tertiary split is visible in the lower half chromosome, and in its half-spindle component.

The sex chromosome, always clearly bipartite through the prophase and first metaphase, does not clearly disclose its secondary split (homologous with the tertiary split of the autosomes) until the onset of anaphasic movement. Herewith becomes apparent the same tendency to independence of chromatid action as characterizes the autosomes. In the upper cell of Fig. 15 one dividing chromatid of the X shows marked elongation and slight stembody formation; the other, little change in shape and a greater growth of stembody. In the X of the lower cell stembody formation seems equally advanced in the two halves, while the elongation factor alone is differentially expressed. This differential behavior emphasizes the reality of the split, which is often suggested but never positively demonstrable in the more synchronously dividing X chromosomes, such as those shown in Figs. 8 and 14.

In the later anaphase of the first division there usually occurs a fusion of the individual stembodies into a single common tube. Herewith the chromosome elements become massed together and their analysis is rendered difficult. The early differences in time and rate of anaphasic separation persist, however, to different degrees in different cells. Late telophase and interkinetic nuclei vary in the degree of dissociation of their chromosome elements from cases of maximum contraction and cohesion, where only five elements are visible (Fig. 6) to cases in which most or all of the ten half chromatids are distinctly separable optically (Figs. 16 and 34).

Chromatid Autonomy in Second Division

In the second division the effective reality of the tertiary split and the independence of action of the chromatids are even more marked than in the first division, due partly, of course, to the smaller number of elements to be analyzed, but partly also to a real intensification of the differential behavior.

In interkinetic and early second metaphase nuclei so compact an association of chromatid halves as is shown in Figs. 6 and 7, which approximate the tribal type, is very rarely encountered. Even in these nuclei, chosen as examples of maximum cohesion of chromosome elements, it will be noted that one autosomal mass in Fig. 6 and the X in Fig. 7 give evidence of subdivision. The vast majority of interkinetic nuclei show a clear spatial separation of the half chromatids. Similarly the re-association of the chromosomal elements for the second division is but rarely a close one. Instead, there occurs an aggregation of the chromosomes of the interkinetic nucleus into three groups—one comprising the two parts of the X, each of the other two the four derivatives of one autosomal chromosome pair. In this re-association into groups we are confronted with a clear case of chromosome pairing operating among multiple elements. The subdivision of the chromosome does not impair the attraction.

The metaphase stage resulting from this re-association or grouping of half chromatids is very transient and seldom established to the point of a precise parallel orientation or plate-like arrangement of the chromosomes. More frequently the loosely assembled four-parted autosomal aggregations seem to pass at once, as soon as contact between their homologous elements is established, into early anaphasic movement. Their half spindles form while the first steps in chromosome separation are under way. The two halves of the X chromosome, now lying in contact or in close proximity, may take any position in the nuclear area. If in the equator, the sex bivalent sometimes shows an elongation in the direction of the long axis of the developing spindles; if extra equatorial, no such change of shape takes place at metaphase. Whatever its position, no spindle is produced by it and it passes with apparent passivity to one pole, with varying degrees of separation apparent between its two components.

In the anaphase each of the two pairs of half chromatids comprising an autosome forms its own individual spindle, often clearly separable from its mate along the plane of the tertiary split. This autonomy of the chromatid, rather than the chromosome as in the tribal type, in spindle formation and anaphasic movement is established by three lines of evidence. First, the half-spindle element of each half chromatid is initially distinct from that of its mate (Figs. 17 and especially 18, where the two chromatid spindles of each chromosome mass are bent at different angles); second, the stembody may be distinct for each chromatid (note the right-hand chromosome in Fig. 20). Even in cases where the two chromatid stembodies seem to fuse centrally, a double region is often apparent distally as in the left-hand spindle of Fig. 24. Third, and most

convincing, the half chromatids of each chromatid separate and pass to the poles at different rates. Again, as in the first division, the two factors of change of shape and of stembody growth may be differentiated. And, as these two factors express themselves to different degrees in the two chromatid spindles of a chromosome, the chromatid autonomy becomes thereby very marked. Thus we may find in a pair of associated dividing chromatids a uniform growth of the stembodies coupled with differing degrees of attenuation of the chromatin masses (see the left-hand chromosome in Figs. 17 and 23). Conversely, differential growth of the two stembodies, coupled with some difference in attenuation, is manifest in both sets of spindles in Fig. 18. As anaphase progresses these differences in rate tend to be maintained or increased. As the two chromatid spindles of a chromosome aggregate elongate, they seem to slide upon one another along the plane of the tertiary division. We have already noted that the stembodies of these two separate chromatid spindles tend to fuse at the equator. Coincidentally each chromatid spindle has undergone a marked elongation; this is evinced by its steady increase in length, decrease in diameter, and compression of the chromatin into flanges along the two walls, both distally and centrally, and in the narrowing and straightening of the half-spindle elements. As this growth in the length of the spindles proceeds, the fusion of the two stembodies noted above becomes complete. The chromatin masses with their separate half spindles are thus brought nearer and nearer together. As long as one pair of separating half chromatids is only slightly in advance of the other, they continue to slide over each other and maintain structural separation (Figs. 22, 23 and 24—the left chromosome aggregate in each case). When the more rapidly moving pair overtakes

PLATES I–IV

All figures are drawn with Zeiss apochromatic 2 mm. obj. and comp. oc. 20, at table level, with Abbé camera lucida, and are reproduced without reduction.

Plate I

Fig. 1. Diploid chromosome group of female. Oogonial mitosis.

Fig. 2. Diploid chromosome group of male. Spermatogonial mitosis.

Fig. 3. Metaphase I viewed from side. Maximum condensation of chromosomes.

Fig. 4. Polar view of metaphase I.

Fig. 5. Early anaphase I, with maximum contraction and synchrony of chromosome elements.

Fig. 6. Interkinetic nucleus—maximum cohesion of elements.

Fig. 7. Metaphase II—maximum cohesion.

Fig. 8. Early anaphase I—with both tetrads showing tertiary split.

Fig. 9. Polar view metaphase I with right-hand tetrad in same position as those of Fig. 8.

Fig. 10. Polar view metaphase I with tetrads in same position as in Fig. 13.

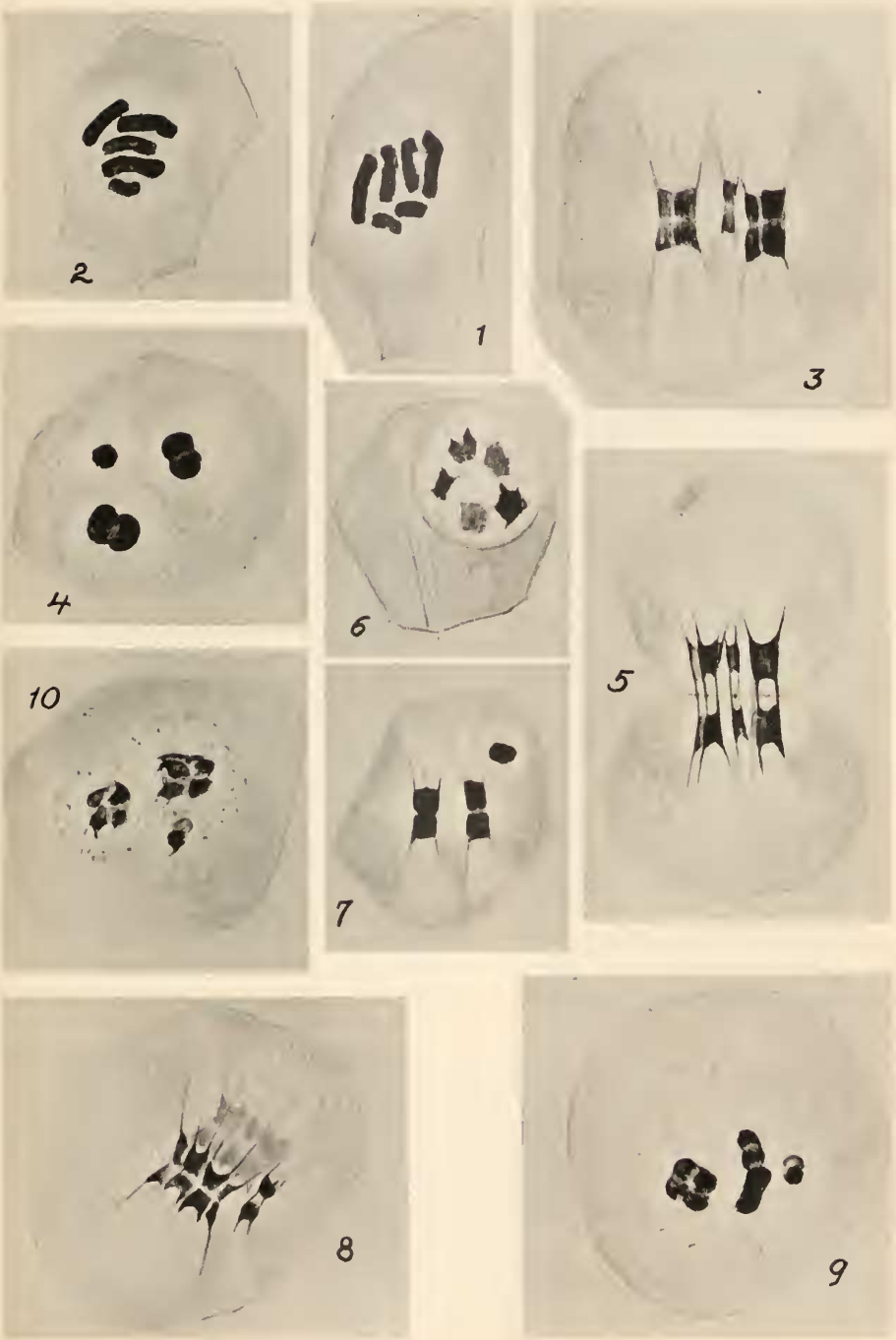


PLATE 1

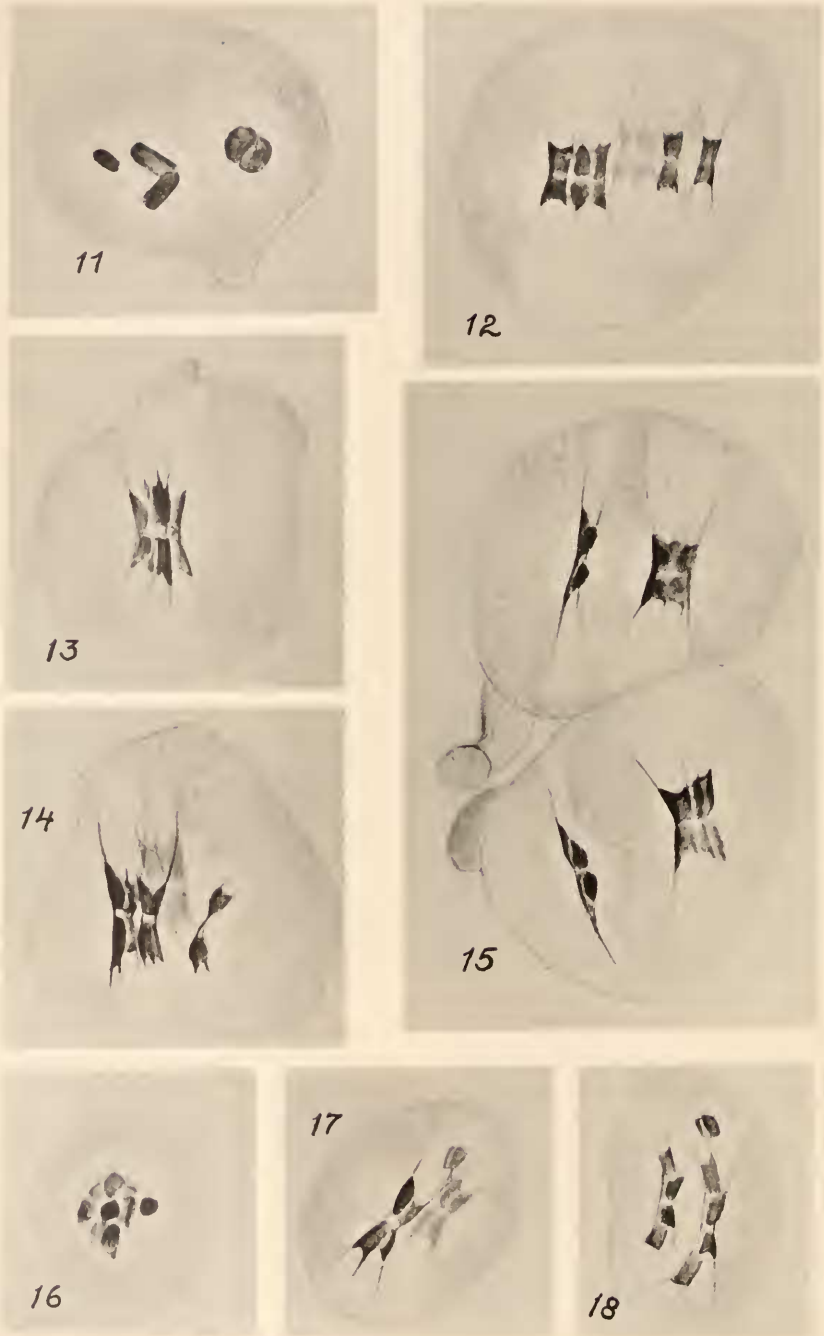


PLATE 2

the slower ones in the passage to the poles, the two partially separated chromatid spindles tend to fuse completely, giving the curious linear aggregates of four chromosome elements within the single spindle tube seen in the left-hand complex of Figs. 20 and 21, and the right-hand complex in Figs. 22, 23 and 24. This fusion of the two originally distinct chromatid spindles into one never involves the substance of the half chromatids but only the sheath material which from its earliest appearance has appeared to be continuous over half spindle, chromatid and stembody. The sex chromosome is often caught up by one or another of the rapidly growing half spindles; it thus often comes to lie within the substance of one half spindle (Fig. 22). When its two components are closely appressed or lie transversely to the long axis of the spindle (Figs. 20, 21 and 25), a linear aggregate of five chromosome elements within a single tubular sheath is produced. The structure thus formed strikingly resembles the metaphase configuration of the second meiotic division in *Protortonia* males (Schrader, 1931).

Late anaphasic movement involves not only continued linear growth of the stembodies but also a continued elongation and attenuation of each chromosome mass itself. This is not surprising, if as I believe, the tubular sheath so clearly demonstrable in optical sections of the stembody (quite as in *Llavcia* spermatocytes), is actually identical with the chromosomal sheath and this in turn continuous with the outer wall of the half spindle. Elongation of a common sheath would force the chromatin masses to elongate. However, some precocious despiralization of the lagging half chromatids probably enters into the picture also; the equatorially directed end of the chromatin mass often becomes indistinct in stain and fuzzy in outline, with considerable indication of a spirally unwinding chromonema. This may begin while the chromatin

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PLATE II

FIG. 11. Polar view metaphase I with left-hand tetrad in the position shown in Fig. 12.

FIG. 12. Metaphase I, lateral view, with the two quartets of each tetrad at right angles to each other.

FIG. 13. Early anaphase I—only one, the larger, tetrad is drawn. Chromatid spindles, and differential anaphasic rates shown.

FIG. 14. Same as 13—with smaller tetrad in early division phase.

FIG. 15. Same as 13—showing chromatid autonomy in the X components. One tetrad omitted in each cell for clarity.

FIG. 16. Interkinetic nucleus showing at least nine separate chromosome elements.

FIG. 17. Early anaphase II, showing reassociation of autosomal derivatives, and chromatid spindles.

FIG. 18. Anaphase II. Chromatid spindles bent. Different rates of anaphasic movement visible in the different chromatid spindles.

is still within the spindle sheath or tube (Fig. 26) and continues steadily throughout the ensuing stages.

The cytoplasmic furrow cuts through at different stages of these anaphasic changes in different cells—but in no case so precociously as in the first division. As the anaphase proceeds the two stembodies approach each other in the midline and fuse into a single common cylinder (Fig. 26). Continuing growth of the stembody and elongation of the chromosomes bring the latter into contact with the cell wall. The chromosomes buckle under; the stembody may extend stiffly into the cell wall (Figs. 28 and 29), then gradually expands as its central end is cut (Fig. 30), and disappears in situ. During these telophasic stages the half chromatids retain their distinct spatial separation and identity,—one telophasic group revealing four half chromatids, all autosomal (Figs. 29 and 30), the others six, four of autosomal and two of sex chromosome origin (Figs. 27 and 28). Despiralization continues throughout these stages, demonstrating conclusively that each half chromatid represents at least one distinct chromonema. Late telophases show a re-association of these distinctly separated half chromatids or chromonemata into pairs. Nuclei in transition from telophase to the resting condition thus show the chromatin in the form of either two or three linear aggregations which tend to be indistinct in fixation and not positively analyzable as to their contained chromonemata, but whose double structure is usually apparent at one or more points.

PLATE III

FIG. 19. Anaphase II, with one pair of half chromatids showing marked elongation.

FIG. 20. Anaphase II. Chromatid spindles separate in right-hand, fusing in left-hand aggregate.

FIG. 21. Same; with fusion of chromatid spindles complete in left-hand aggregate.

FIG. 22. Same; right-hand aggregate shows quarternary split.

FIG. 23. Stages in fusion of spindles, and marked autonomy in anaphasic rates.

FIG. 24. Same; left-hand aggregate shows quarternary split in one pair of half chromatids.

FIG. 25. Late anaphase II; complete fusion of chromatid spindles forming two linear aggregates.

FIG. 26. Same; despiralization visible in lagging half chromatids.

FIGS. 27 and 28. Telophase II, showing continued despiralization. Six elements visible, four autosomal and two X chromosomal.

FIGS. 29 and 30. Late telophase II—with early stage in reassociation of half chromatids. These nuclei carry no sex chromosomes.

FIG. 31. Early binucleate spermatid, formed by fusion of products of second division. Shows reassociation of half chromatids into two and three threads respectively.

PLATE 3

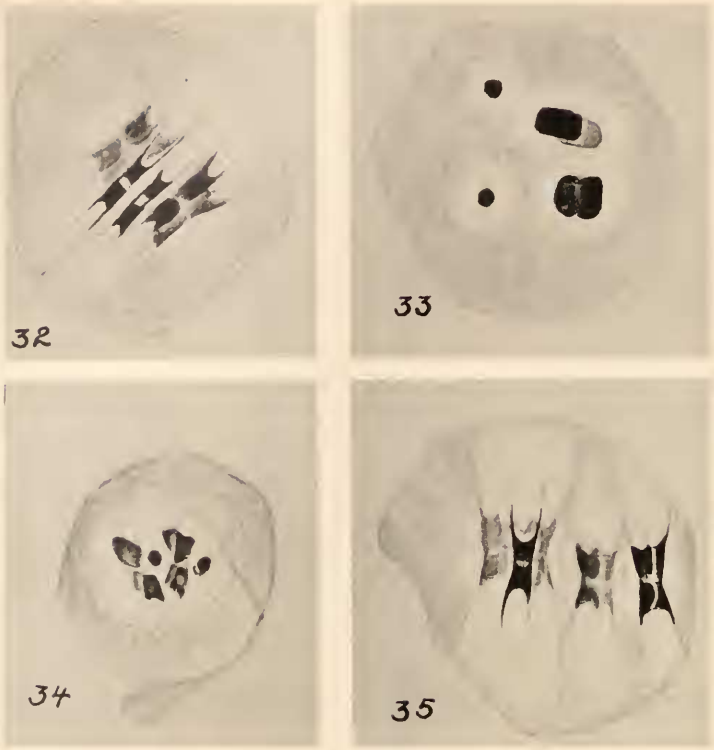

PLATE 4

FIG. 32. Early anaphase I, showing two X chromatids each with its own spindle and dividing independently.

FIG. 33. Polar view of metaphase I with the two X chromatids completely separated.

FIG. 34. Interkinetic or early prophase II with X half-chromatids separated and in one case double.

FIG. 35. Metaphase I—in which the small autosomes, the two right-hand chromosomes, have developed asynaptically and are dividing independently.

Dissociation of the Chromatids of the X Chromosome

This phenomenon may logically be considered as an extreme expression of the chromatid autonomy described in the foregoing section. I separate it to emphasize its bearing on special problems of sex chromosome behavior. The X chromosome, normally double longitudinally throughout the prophase of the first meiotic division, is occasionally found to have separated into its two components. It then appears as two spatially distinct bodies—each measuring half the diameter of the normal X (Fig. 33). This separation of the two chromatids of the X may be traced back, as in the case of the asynaptic autosomes, to the formation of two separate prophase vesicles, in each of which one chromatid of the X evolves. Again, as in the case of the asynaptic autosomes, the subdivision of the mass favors its optical resolution and it is frequently easy to demonstrate that each of these dissociated equational X chromatids is itself double. With the dissolution of the vesicle walls this cleavage, secondary for the X but homologous with the tertiary split in an autosome, becomes very marked. Each bipartite chromatid develops its own half-spindle element and divides independently of the other. In Fig. 33 the wide spatial separation of the independent X chromatids in an early first metaphase nucleus is shown. The vesicle walls have just disappeared, but the nucleoplasm is still visibly demarcated from the cytoplasm. Figure 32 shows the formation of a spindle by each chromatid and the beginning of the anaphasic separation of the half chromatids. It would seem probable that the original dissociation of the X chromatids occurs along the primary split—always so early and clearly evident in the first prophase nuclei. If this be true, the independent division of the dissociated chromatids takes place along the plane of the secondary (= tertiary for autosomes) split. Surely no more final demonstration of the effective reality of this split could be asked. The only alternative assumption is to suppose that the primary dissociation of the X chromatids occurs along the secondary split. Even on this assumption the development of the two chromatids in separate vesicles, the production of individual spindles by each of them, and finally their independent division, form an equally conclusive demonstration of the effectiveness of the tertiary split.

The dissociation and independent division of the chromatids of the X in no way interferes with the normal course of reduction. Telophases of the first division may show a wide spatial separation of the two X components (Fig. 34); but these invariably re-associate during the orientation of the chromosomes for the second metaphase, and come to lie either in contact or very close together (Figs. 17 and 18). Together

they then pass to one pole during the ensuing anaphasic movements. In no case out of several hundred second anaphases analyzed, was there a failure of this re-association and reduction.

Asynapsis

As noted above, the first meiotic prophase is initiated, in those cells conforming to the tribal pattern, by a lobulation of the nucleus into three distinct vesicles proportionate in size to the chromosome pairs. The smallest of these vesicles contains the sex chromosome; each of the other two, one pair of autosomes. Within the vesicle the autosomes pass through their synaptic and diakinetic phases. The vesicle walls persist until the chromosomes have reached their maximum contraction, and disappear while the half spindles are forming preparatory to metaphase orientation. Now in a certain percentage of spermatocytes, in all males studied, the members of one or occasionally of both pairs of autosomes develop in separate vesicles and hence completely without any synapsis.[2] Each chromosome develops its own spindle and divides in the first division entirely independently of its mate. The bearing of this phenomenon on the question of the tertiary split lies in the fact that the spatial separation of the two homologous chromosomes, ordinarily so closely associated in the tetrad, renders the quadripartite structure of each first metaphase chromosome unequivocally clear. Figure 35 presents a first metaphase in which the two smaller autosomes have developed asynaptically and are dividing independently of each other. The tertiary split is sharply visible in both. The smaller number of separate elements, compared with the tetrad, and the associated tendency toward a greater separation between them, further preclude any confusion between the tertiary split and that appearance of terminal bifurcation caused by the collar-like flange developed on each half chromatid with the growth of its half spindle.

Evidence for a Fourth Split

There is occasional scattered evidence for still another subdivision of the meiotic chromosome of *Llaveiella*. Thus in a telophase following the independent division of dissociated X chromatids, a clear bifurcation of one of the X components was encountered in three cases (Fig. 34, right-hand X). In the autosomes also a fourth split is occasionally

[2] As in *Llaveia bouvari*, it is the smaller pair of autosomes that shows a regularly high incidence of asynapsis. In *Llaveiella* the number of spermatocytes showing asynapsis of this pair ranges from 2.4 to 8.7 per cent. A comparative study of the asynaptic phenomena in three species of Llaveiini and their significance for theories of meiosis is under way at present.

clearly demonstrable. Not infrequently, during the differentially progressing anaphasic movements in the second division, one notes a partial subdivision of one pair of the half chromatids. In several nuclei this division was so complete and so emphasized by the different poleward rates of the quarter chromatids involved as to offer quite conclusive evidence for the reality of the fourth split. Thus in the right-hand spindle in Fig. 22 six well separated chromosome elements are visible. The spindles of the two pairs of separating half chromatids of one autosomal group have here coalesced into a single tube; one pair of the contained half chromatids has divided again, and its halves are moving poleward at different rates,—thus giving six elements in a linear aggregation. Upon careful focusing the two terminal elements in this chain appear to have greater mass than the others; it is thus probable that it is the slower, more centrally placed pair of half chromatids which has redivided. However, all these elements are so small that attempts at accurate measurement proved futile. That these six elements are all derivatives of the one pair of autosomes involved is clear from the presence of the two X chromatids in the half spindle of the other autosomal aggregate. Again in the left-hand spindle in Fig. 24 one pair of the separating half chromatids is again clearly subdivided, with one pair of derivatives well in advance of the other in its poleward movement. In this case the spindles of the two chromatids involved are fused only in the stembody region, so that it is here the more precociously dividing chromatid that shows the fourth split.

Discussion

Number of Chromonemata per Chromosome

What is the bearing of these divisions of the chromosome along the tertiary and even quarternary splits on the question of the actual duplication of chromonemata, and their number per chromosome at a given point in the meiotic cycle? It is clear that the divisions here described involve the whole chromosome—sheath, matrix and contained chromonemata. The techniques employed do not permit a differentiation of chromonemata within the chromosome unit at all stages. In the telophase of the second division, however, each visible subdivision of a chromosome presents the appearance of a coiled thread, progressively elongating by despiralization. In these stages a one-to-one relation between the chromosome unit as visibly differentiated throughout the cycle, and its chromonemal content is indicated. But even without this evidence, it is obvious that the complete cleavages of the chromosome into chromatids, half chromatids and quarter chromatids as seen in the

Llaveiella material do give us a minimum limiting value for the number of chromonemata present. Obviously each subdivision must contain at least one chromonema. The meiotic chromosome thus comprises at least four chromonemata at metaphase—at least two in anaphase and telophase. The occasional clear separation along the quarternary split would imply that this minimum value is below the actual value—that the metaphase chromosome is at least eight-parted, the anaphase and telophase chromosome, four-parted. Such values would agree with the findings of Nebel (1932, 1933*a* and *b*, 1936, 1937), Nebel and Ruttle (1936, 1937), Stebbins (1935) and Goodspeed, Uber and Avery (1935). In *Llaveiella*, however, the evidence for the regular occurrence of these higher values is not conclusive. The quarternary split, even if we assume it to be a constant feature, is here very rarely followed by a complete cleavage of the chromosome, and hence is only rarely demonstrable. For a minimum value of two chromonemata per chromosome at the lowest point in the cycle, on the other hand, the evidence seems incontrovertible, and for the following reasons. Cleavage of the chromosome along the tertiary split occurs in the majority of the spermatocytes. Moreover, the completeness of the cleavage and the actual movement of the chromosome elements along its plane preclude cytological misinterpretation. Darlington condemns as unreliable much of the earlier evidence for the tertiary split because it depends on the detection of longitudinal doubleness in a cylinder which he claims is less than one half the wave length of the light used. This objection ignores, as Kaufmann (1936) has pointed out, all the evidence from widely diverging chromosome ends, split satellites, and end views of chromonemata. Moreover, it certainly cannot apply to the *Llaveiella* case, for here we are not dealing with the admittedly difficult differentiation of coiled threads intimately associated in a common cylinder. Rather the *Llaveiella* evidence rests on complete cleavages of the whole chromosome into units whose spatial separation and individual behavior preclude optical illusion in their interpretation.

Chromosome Pairing

These data also permit certain conclusions on the forces involved in chromosome pairing. The mutual attraction of homologous chromosomes prior to their reductional separation is here demonstrated to be quite independent of the subdivision of the chromosome, and hence of the multiplicity of chromonemata. In the interkinetic nucleus ten spatially separate and distinct half chromatids may be distinguished. At the prophase of the second division these units re-associate according to their derivation into three groups—one of which contains the two deriva-

tives of the X chromosome, each of the other groups the four derivatives of one pair of autosomes. The force that brings these together is clearly independent of the subdivision of the original chromosomes.

Furthermore the data demonstrate once again that this association is also independent of synapsis and of the formation of chiasmata. This is shown by the fact that the two chromosomes constituting one or both pairs of autosomes may pass through the meiotic prophases in separate vesicles without any possibility of synapsis or of crossing-over, may each divide independently of its mate—and still the derivatives of the two homologous chromosomes will come together in brief contact prior to their reductional separation in the second division. This independence between the attraction factor on the one hand and synapsis and chiasmata formation on the other obtains also in *Llaveia* (Hughes-Schrader, 1931) and reaches complete expression in *Protortonia* (Schrader, 1931).

Furthermore, this attraction operates between strictly equational derivatives of one chromosome, as well as between derivatives of homologous chromosomes. This is attested by those cases in which the two chromatids of the X chromosome have been completely separated during the first division. It will be recalled that occasionally the equational halves of the X evolve in separate prophase vesicles, each develops its own spindle, and divides quite independently of its mate. In such cases the half chromatids of the X may be widely separated in the interkinetic nucleus, yet they always re-associate prior to the second division. It may be added, although it raises another question, that the passivity of the X in the second division, expressed in its failure to produce a spindle, is also seen to be independent of its subdivision.

Evolutionary Relationships

The tribe Llaveiini is recognized on both cytological and more orthodoxly systematic grounds (Morrison, 1928; Schrader, 1930; Hughes-Schrader, 1931) as the most primitive unit of the most generalized subfamily and family of the Coccidae. The *Llaveiella* case supports this conclusion and in addition throws some light on the evolutionary interrelationships within the tribe Llaveiini. Of the three genera of this tribe thus far studied—*Llaveia, Protortonia,* and *Llaveiella, Llaveia* presents the basic or ancestral type of male meiosis, primitive in the high degree of retention of synapsis, specialized in its compound chromosomally derived division figure, and in the incidence of the asynaptic habit. *Llaveiella* retains this basic pattern sufficiently to demonstrate a close but derivative relationship. It adds (1) an increased expression of the asynaptic habit, (2) chromatid, rather than chromosome, autonomy

in the formation of the compound chromosomal division figure and in the anaphasic movements of the chromosome elements, (3) the subdivision of the chromosomes along the tertiary and occasionally even the quarternary split, and (4) a tendency for the separation and independent division of the equational halves of the X chromosome.

Without question the genus *Protortonia* presents the most highly specialized conditions in the group. I have elsewhere discussed its probable derivation from a *Llaveia*-like pattern (Hughes-Schrader, 1931). The *Llaveiella* case is of especial interest in this regard because it suggests some of the changes that may have been instrumental in the evolution of the *Protortonia* meiosis.

Take first the question of asynapsis. Incipiently developed in *Llaveia,* where the two shorter autosomes evolve in separate vesicles and divide independently in a small percentage of the primary spermatocytes, this procedure is definitely more marked in *Llaveiella.* Here the asynaptic habit characterizes the smaller pair of autosomes in a larger percentage of the cells, and occasionally also involves the other pair of autosomes. In *Protortonia* this asynapsis is completely established as regular procedure for all chromosomes—the only remnant of synaptic behavior being the retention of a single vesicle for one pair of autosomal derivatives in the first prophase. The complete expression of asynapsis in this related genus supports the idea that its partial expression in *Llaveia* and *Llaveiella* has also a genetic basis, and is significant for the direction of evolution within the group.

Again, the complete subdivision and separation of the equational halves of the first meiotic prophase chromosomes, so unique a feature of the *Protortonia* cycle,—is foreshadowed in *Llaveiella.* In the latter case only the X chromosome is involved, and that only in a very small number, approaching 1 per cent, of the cells. A unique feature of the *Llaveiella* case is the independent division of the separated X chromatids —a feature probably correlated with the strong expression of the tertiary split (here effective in division) in *Llaveiella* and its absence in *Protortonia.*

To me the most suggestive application of the *Llaveiella* data to the problems of the *Protortonia* meiosis involves the possible origin of the strange linear aggregate of chromosomes in the spindle of the second division of the latter genus. It will be recalled that in *Protortonia* the five chromosomes of each interkinetic nucleus formed short chains of two and three elements each. These chains then become appressed together, the chromosomes of one slipping into the interstices of the other chain in such a way that there results a single linear aggregate of five elements lying within a common tube. From the terminal chromosomes

of this chain delicate half spindles are formed. Anaphase movements then separate the chain into one group of three, always including the X chromosome, and one of two chromosomes. The tubular stembody involved in this anaphasic movement, together with the half spindles, demonstrate its essential likeness to the meiotic spindles of *Llaveia* and *Llaveiella*.

I would suggest that the two short chains formed from the interkinetic chromosomes in *Protortonia* are to be interpreted as precocious anaphase spindles; that the "fibers" (tubes) connecting them are precociously formed interzonal connectives (stembodies); and finally, that the different distances separating the chromosomes within the two chains represent differences in rate or time of start of the anaphasic chromosome movements. Ordinary prophase and metaphase would thus be considered as entirely eliminated in the second division in *Protortonia* (in this respect compare *Llaveiella* where such a process is well under way). That one of the two chains in *Protortonia* often contains three chromosomes means simply that the X (more or less passive in the second division) has been caught up in one spindle as it forms,—and may thus occupy either a terminal or a central place within the group. The passivity of the X, unless or until caught up into one of the developing spindles, is shown in Schrader's figures 26 and 27. The analogy with *Llaveiella* may be pursued further. Just as in *Llaveiella* the separate spindles of the two chromatids, whose chromosomal elements are separating at different rates, often tend to slip over one another and actually to fuse into a single tubular spindle containing four (or five or six if the X be involved) chromosome elements in linear order—so, too, in *Protortonia* the single chain of five may be formed by the fusion of the two differentially developed precocious anaphase spindles. In *Llaveiella*, of course, the chromatid is the unit in the differential development of the spindles, while in *Protortonia* the whole chromosome assumes this rôle. Further support for this concept of differential anaphase rates in *Protortonia* is found in the first division. It will be recalled that here the separate equational halves of the X and of each of two of the autosomes do actually form their individual spindles at slightly different times and rates. The behavior of the other two chromosomes in the first division in *Protortonia* is not so easily analyzed on this hypothesis. The four equational halves of this pair of chromosomes evolve in a single, instead of in separate vesicles, and they take up a linear alignment within the vesicle before any spindle forms between or involving them. When the spindle does form, however, just as the vesicle walls disappear, it shows the same fundamental structure and arrangement and behavior of its chromosomal elements as does the linear ag-

gregate in the second division of *Protortonia* and *Llaveiella.* This linear alignment preliminary to spindle formation emphasizes the reality of that extrachromosomal force postulated by Schrader in his analysis of the *Protortonia* figure. This force is expressed in the elongation and terminal attenuation of the prophase vesicles and in their convergence to polar centers (Schrader's figures 13 and 14), and would seem to provide an adequate causal factor for the linear aggregation of the chromosome units. That this force is interacting even here with one of intrachromosomal nature is indicated by the differential rate of spindle formation and anaphasic movement characterizing the first division chromosomes. In the formation of the single linear aggregate of the second division, and in the similar configurations in *Llaveiella,* on the other hand, there is a lessened expression of the extra-chromosomal force (thus no vesicle pressure, no convergence to polar centers), and the intrachromosomal forces are hence more obvious. Even here, however, the extrachromosomal polarizing force may well be the cause of the close appression and actual fusion of the two originally separate anaphasic spindles. The balance between the intra and extrachromosomal factors is thus seen to be characteristically different in the three genera available for comparison. In *Llaveia* the extrachromosomal force is weak (witness the complete absence of polar centers, the delay and incomplete metaphase orientation of spindles); the intrachromosomal forces making for linear aggregations (chromatid autonomy and differential anaphasic rates) are also not active; the resultant is the complete absence of linear arrangement. In *Llaveiella,* too, polarization is weak (the fusion of spindles being its only obvious expression), but the divergent rate factor is strong. The result is frequent but variable formation of linear aggregates. Finally in *Protortonia* both factors are operative to a high degree, with a consequent constant formation of linear aggregates.

The Normal Range of Variation in the Meiotic Mechanism

The unparalleled diversity of meiotic phenomena presented by male coccids forces us to keep our working hypotheses of the mechanisms involved flexible. The retention by the females in each species thus far studied of an orthodox meiotic behavior, while the males of the different groups have developed their amazing range in method, is evidence that we are dealing here with successful, workable modifications of the common mechanism—not with anomalies or abnormalities lying without the law. The justness of this point of view receives striking confirmation in the case of *Llaveiella taenechina.* Here within a single individual

sister cells vary in such supposedly basic attributes as the presence or absence of synapsis, the occurrence or omission of chiasmata, in the rate and incidence of anaphasic movements, in the time relations of chromosome division. Indeed individual chromosomes within a single cell are seen to vary in respect to these important particulars. And yet in all the variants a normal and regular reduction is consummated. It seems, therefore, that these variant meiotic procedures, going on side by side in the same testes with the tribal type of meiosis, and in the same species with a perfectly orthodox meiosis in the female line must be admitted as falling within the normal range of variation of the meiotic mechanism. The evidence they offer is applicable to and must be taken into account in any general theory of meiosis.

Summary

1. The diploid chromosome set of the female *Llaveiella taenechina* comprises three pairs of rod-shaped chromosomes distinguishable by size. The shortest one is unpaired in the male, giving a diploid set of 5.

2. The chromosome behavior and achromatic figure are normal in the female cycle and in the male somatic and spermatogonial mitosis. Meiosis in the male conforms to the *Llaveia* type, but only a minority of the spermatocytes adhere strictly to this scheme. The majority of the cells show different combinations of four major types of variation, all of which are successful in sperm formation. The variant procedures may be summarized as follows.

(*a*) Chromatid autonomy. The chromosomes are subdivided along the tertiary split. Each pair of half chromatids produces an individual spindle. Anaphasic movements may start at different times and proceed at different rates in the four chromatid spindles. In the second anaphase the two chromatid spindles of an autosomal group may fuse to form a linear aggregate of chromosomal elements in a single tubular spindle.

(*b*) The complete dissociation of chromatids. The two chromatids of the X chromosome may evolve in separate prophase vesicles and divide independently of each other in the first division. The re-association of their derivatives and their common passage to one pole in the second division is not thereby affected.

(*c*) Asynapsis. Each member of one or both pairs of autosomes may evolve in a separate prophase vesicle, without synapsis and chiasma formation, produce an individual spindle, and divide independently of its mate in the first division. Chromosome pairing for the second division is not thereby affected.

(*d*) Quarternary split. Occasionally one or more chromosomes may be further subdivided along the fourth or quarternary split.

Conclusions

1. The multiple structure of the meiotic chromosome is established. The complete cleavage of the chromosome along the tertiary split gives a minimum value of two chromonemata per chromosome at the lowest point in the cycle.

2. The force effective in chromosome pairing is independent of the subdivision of the chromosome and hence of the multiplicity of chromonemata.

3. The meiotic association of homologous chromosomes is independent of prophase synapsis and of chiasma formation.

4. Chromosome pairing operates between strictly equational derivatives, as well as between those from different homologous chromosomes.

5. The passive behavior of the sex chromosome in the second division—its failure to produce a spindle and undergo anaphasic separation,—is independent of its subdivision.

6. The data support the theory that at least two forces operate in anaphasic movement, one intrinsic to the chromosome, and one expressed in stembody growth.

7. Linear aggregates of chromosomal elements in a single tubular spindle may arise by fusion of individual chromatid spindles.

8. The evolutionary relationships within the tribe Llaveiini are discussed with especial reference to the meiotic division figure of *Protortonia*.

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